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Genetic screening and evaluation for chromosomal abnormalities of infertile males in Jilin Province, China

M. Zhang, H.-T. Fan, Q.-S. Zhang, X.-Y. Wang, X. Yang, W.-J. Tian and R.-W. Li

Andrology Laboratory, Department of Urology, The Second Hospital of Jilin University, Changchun, Jilin Province, China

Corresponding author: M. Zhang
E-mail: zhangming1982jdey@126.com

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ABSTRACT. Chromosomal abnormality is the most common genetic cause of male infertility, particularly in cases of azoospermia, oligozoospermia, and recurrent spontaneous abortion. Chromosomal rearrangement may interrupt an important gene or exert position effects. The functionality of genes at specific breakpoints, perhaps with a specific role in spermatogenesis, may be altered by such rearrangements. Structural chromosome abnormalities are furthermore known to increase the risk of pregnancy loss. In this study, we aimed to assess chromosomal defects in infertile men from Jilin Province, China, by genetic screening and to evaluate the relationship between structural chromosome abnormalities and male infertility. The prevalence of chromosomal abnormalities among the study participants (receiving genetic counseling in Jilin Province, China) was 10.55%. The most common chromosome abnormality was Klinefelter syndrome, and the study findings suggested that azoospermia and oligospermia may result from structural chromosomal abnormalities. Chromosome 1 was shown to be most commonly involved in male infertility and balanced chromosomal translocation was identified as one of the causes of recurrent spontaneous abortion. Chromosomes 4, 7, and 10 were the most commonly involved

chromosomes in male partners of women experiencing repeated abortion.

Key words: Chromosomal abnormality; Male factor infertility; Azoospermia; Oligozoospermia; Recurrent spontaneous abortion

INTRODUCTION

Chromosomal abnormalities are an important cause of male factor infertility. The rate of chromosomal abnormality in infertile men is 2.1-19.48% (Yatsenko et al., 2010; Fu et al., 2012; Ghorbel et al., 2012; Quan et al., 2013; Ananthapur et al., 2014; Kate et al., 2014). Chromosomal defects may decrease male fertility and increase the history of female adverse fertility outcomes, e.g., recurrent spontaneous abortion or stillbirth.

Sex chromosome aneuploidies and structural chromosome abnormalities are usually associated with male factor infertility. Klinefelter syndrome is the most common genetic cause of male infertility and primary testicular failure. Carriers of chromosomal rearrangement can be phenotypically normal, unless one of the translocation breakpoints interrupts an important gene or the rearrangement exerts a position effect (Harton et al., 2012). Structural chromosomal abnormalities thus result in an increased risk of pregnancy loss or transmission of chromosomal abnormalities to offspring due to the production of a higher number of unbalanced spermatozoa (Godo et al., 2013).

In the present study, we report the outcome of genetic screening for chromosomal defects in men with infertility from Jilin Province, China, and evaluate the relationship between structural chromosomal abnormalities and male infertility.

MATERIAL AND METHODS

Patients

From January 2009 to December 2014, 3319 patients were evaluated during male genetic counseling at the Clinic of Andrology, Department of Urology, Second Hospital of Jilin University. Based on their clinical characteristics, all subjects were classified into one of three categories: male infertility for non-conception (1), adverse fertility history for recurrent spontaneous abortion or stillbirth (2), or pre-pregnancy genetic counseling (3).

Cytogenetic analysis

Blood samples from all patients (18-46 years old) were collected for routine cytogenetic analysis. Peripheral blood (0.5 mL) was collected in sterile tubes containing 30 U/mL heparin and G-banding was performed using cultured peripheral blood lymphocytes (Zhang et al., 2013). At least 20 metaphases were analyzed per patient and chromosomal abnormalities were described according to the International System for Human Cytogenetic Nomenclature (2009).

Semen analysis

Semen analysis was performed according to the procedures recommended by the World Health Organization guidelines (World Health Organization, 1999), in which AS describes cases in which no sperm were present during semen analysis or upon centrifugation and pellet analysis, OS describes cases with sperm densities between 0.5 x 10^6/mL and 5 x 10^6/mL, and sOS describes cases in which sperm density is less than 0.5 x 10^6/mL.

RESULTS

A total of 3319 males were included in this study. Conventional cytogenetic analysis was carried out and revealed chromosomal abnormalities in 350 (10.55%) of the study participants. Of the 350 patients with an abnormal karyotype, men with infertility, men with adverse fertility history, or men undergoing pre-pregnancy genetic counseling made up 240 (68%), 58 (17%), and 52 (15%) cases, respectively.

Among the infertile men with chromosomal abnormalities, 164 (68%) of the 240 cases had numerical chromosomal abnormalities, 34 (14%) had chromosomal polymorphism, 28 (12%) had structural chromosomal abnormalities, 10 (4%) had chromosomal mosaicism, and 4 (2%) had complete sex reversal of XX (46,XX) (Figure 1a).

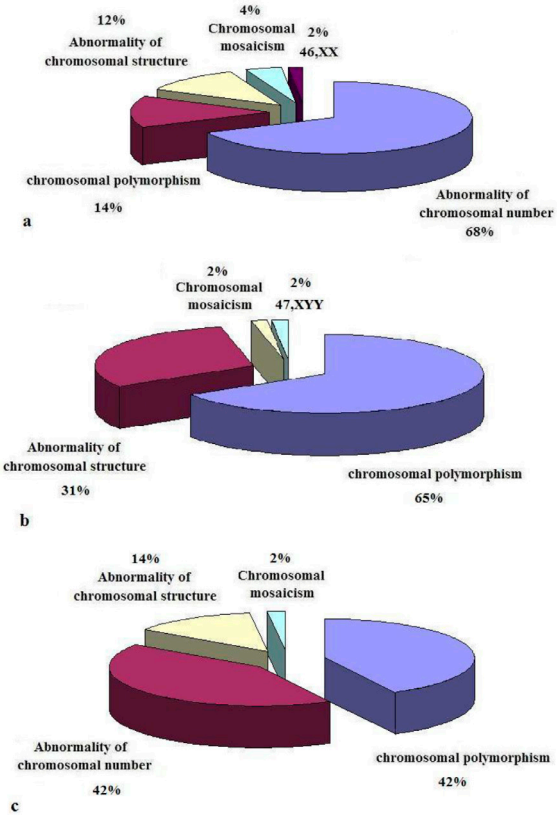

Figure 1. Type and frequency of chromosomal abnormalities in infertile males undergoing genetic counseling for male infertility for non-conception (**a**); adverse fertility history for recurrent spontaneous abortion or stillbirth (**b**); or pre-pregnancy (**c**).

The 164 cases of numerical chromosomal abnormalities included 159 men with nonmosaic Klinefelter were included. The results of karyotype and semen analyses of the 28 cases of structural chromosomal abnormality are shown in Table 1. The patients exhibiting structural sex abnormalities were found to have azoospermia and chromosomes 1 (N = 10) and 13 (N = 6) were most commonly found to be involved on these abnormalities.

Table 1. Karyotype and semen analysis results for infertile male.

Pt No.	Results	Semen Analysis
1	46, Y, t(X;2)(p22;p11)	AS
2	46, X, t(Y;4)(p11;p14)	AS
3	46, X, t(Y;14)(q11;p11)	AS
4	45, X, t(Y;13)(p10;q10)	AS
5	45, X, t(Y;15)(p10;q11)	AS
6	46, XY, t(1;2)(q21;p23)	OS
7	46, XY, t(1;2)(q21;q37)	OS
8	46, XY, t(1;3;6)(p32;q29;q14)	AS
9	46, XY, t(1;4)(p36;q31)	sOS
10	46, XY, t(1;9)(p32;p24)	OS
11	46, XY, t(1;12)(q42;q13)	sOS
12	46, XY, t(1;13)(p22;q14)	OS
13	46, XY, t(1;18)(p32;q23)	OS
14	46, XY, t(1;20)(p13;p11.2)	sOS
15	46, XY, t(7;15)(p15;q15)	OS
16	46, XY, t(10;13)(q10;q10)	OS
17	46, XY, t(11;22)(q25;q13)	Asthenospermia
18	45, XY, der(13;14)(q10;p10)	OS
19	45, XY, der(13;21)(q10;q10)	sOS
20	45, XY, der(14;15)(q10;q10)	OS
21	45, XY, der(14;21)(p10;p10)	OS
22	45, XY, der(14;21)(q10;q10)	OS
23	46, XY, inv(1)(p36q25)	sOS
24	46, XY, inv(3)(p21q25)	OS
25	46, XY, inv(4)(p14q21)	sOS
26	46, XY, inv(11)(p15q12)	OS
27	45, XY, dic(17;22)(p13;q13)	sOS
28	46, XY, dic(13;19)(p11;q12)	OS

AS: azoospermia; OS: oligozoospermia; sOS: severe OS.

In men with adverse reproductive histories, 39 cases (65%) were identified as chromosomal polymorphism, 18 (31%) as structural chromosomal abnormality, 1 (2%) as chromosomal mosaicism, and 1 (2%) as 47, XYY karyotype (Figure 1b). Karyotype and miscarriage data for the 18 cases of structural chromosomal abnormality are shown in Table 2. In these cases, chromosomes 4, 7, and 10 were identified as the most commonly involved chromosomes (N = 4).

Table 2. Karyotype and miscarriage data for male partners with recurrent spontaneous abortion.

Pt No.	Results	Number of miscarriages
1	46, XY, t(1;10)(p31.2;q26)	4
2	46, XY, t(3;7)(p23;q21.2)	3
3	46, XY, t(4;14)(q25;q24)	3
4	46, XY, t(4;21)(q21;q12)	3
5	46, XY, t(4;5)(q21;p15)	3
6	46, XY, t(4;9)(q35;p13)	2
7	46, XY, t(6;7)(q15;p15)	4
8	46, XY, t(6;8)(p21;q24)	4
9	46, XY, t(6;9)(q26;p13)	3
10	46, XY, t(7;10)(q32;q21)	2
11	46, XY, t(7;8)(q32;q22)	3
12	46, XY, t(9;15)(p14;q22)	2
13	46, XY, t(10;21)(p11;q22)	2
14	46, XY, t(10;22)(q25;q13)	3
15	45, XY, der(13;14)(q10;q10)	2
16	45, XY, der(13;14)(q10;q10)	3
17	45, XY, der(15;21)(q10;q10)	2
18	46, XY, t(18;20)(p11;q11)	2

Of the men undergoing pre-pregnancy genetic counseling, 22 (42%) exhibited chromosomal polymorphism, 22 (42%) had numerical chromosomal abnormalities, 7 (14%) had structural chromosomal abnormalities, and 1 (2%) had chromosomal mosaicism (Figure 1c). Of the 22 men with numerical chromosomal abnormality, 21 were diagnosed with nonmosaic Klinefelter syndrome.

DISCUSSION

Infertility affects 15% of couples who attempt pregnancy and male infertility is the cause in half of all childless couples. Chromosome analysis is a valuable tool for the diagnosis of male infertility and chromosomal defects are the most common genetic abnormalities in infertile men, with an incidence of cytogenetic abnormalities ranging from 2.1 to 15.5% (Ananthapur et al., 2014).

Differences in chromosomal defect incidence have been reported across different regions: Yatsenko et al. (2010) reported the overall incidence of chromosomal abnormalities to about 8.2% in 5-year study of cytogenetic aberrations in American infertile men, while 14.3% of infertile males tested by karyotyping were found by the Mayo clinic cytogenetics laboratories to have chromosomal abnormalities (Hofherr et al., 2011). Various numerical and structural chromosome abnormalities were identified in 19.48% of infertile Tunisian men with poor semen quality (Ghorbel et al., 2012) and chromosome analysis revealed major chromosome abnormalities in 10.2% of infertile men in the Indian population (Kate et al., 2014). Karyotype abnormalities were identified in 7.2% of infertile males in Turkey (Cavkaytar et al., 2012) and the total prevalence of chromosomal abnormalities was found to be 4.3% (5/115) in Isparta (South of Turkey) (Kosar et al., 2010). Pylyp et al. (2013) reported chromosomal abnormalities in 17% of Ukrainian patients with sperm disorders and Fu et al. (2012) reported 11.55% of infertile Chinese men to have chromosomal abnormalities. The rate of chromosomal anomaly in men living in Central China was furthermore reported to be 6.84% (Liu et al., 2013) and abnormal chromosomal karyotypes were identified in 8.84% of infertile northeastern Chinese men (Li et al., 2012) and in 7.61% of male partners in Sichuan Province, China (Quan et al., 2013). In this study, the total prevalence of chromosomal abnormalities among the study participants was found to be 10.55% and the incidence of numerical or structural chromosomal abnormality was 7.68% (255/3319).

A high prevalence of chromosomal abnormalities was observed in the infertile men with severe oligozoospermia or non-obstructive azoospermia. Mafra et al. (2011) reported chromosomal abnormalities for 6.2% of infertile Brazilian men with severe oligozoospermia or non-obstructive azoospermia and the total prevalence of chromosomal abnormalities was found to be 11.2% in infertile males with oligozoospermia and azoospermia in southeast Turkey (Balkan et al., 2008). Ocak et al. (2014) reported structural or numerical chromosomal abnormalities to be present in 12% of patients with azoospermia or severe oligospermia and 10.9% of Chinese patients with azoospermia or severe oligozoospermia were found to have chromosomal abnormalities (Zhou-Cun et al., 2006).

The most common chromosomal abnormality identified in this study was Klinefelter syndrome. Ghorbel et al. (2012) reported that Klinefelter syndrome accounts for 66.7% of cytogenetic defects and Amouri et al. (2014) furthermore reported that of 52 patients with abnormal cytogenetic results, Klinefelter syndrome was observed in 71% of patients. In this study, Klinefelter syndrome was observed in 51.4% (180/350) of the chromosomal abnormality cases.

Structural chromosomal abnormalities are an important cause of male infertility. Chromosomal rearrangement may interrupt an important gene or alternatively may exert a position

effect (Harton et al., 2012). These rearrangements may alter the functionality of genes at specific breakpoints such as those with a specific role in spermatogenesis. This may cause defective spermatogenesis, resulting in the abnormalities observed in semen analyses (Ching et al., 2012; Olesen et al. 2001). In this study, five patients with structural sex abnormalities exhibited showed azoospermia and only one patient with 46, XY, t(11;22)(q25;q13) had asthenospermia. Other structural abnormalities were identified as OS or sOS. Chromosome 1 was the most commonly involved chromosome and may therefore contain genes related to spermatogenesis.

Recurrent spontaneous abortions are due to chromosomal rearrangement and structural chromosome abnormalities increase the risk of pregnancy loss or transmission of chromosomal abnormalities to offspring due to the production of a higher number of unbalanced spermatozoa (Godo et al., 2013). Kochhar et al. (2013) reported that among 16 male carriers of a structural chromosomal rearrangement in couples experiencing recurrent miscarriage, chromosome 4 was most frequently found to be involved. In this study, 18 carriers of balanced translocation (Table 2) experienced repeated abortion. Chromosomes 4, 7, and 10 were found to be the most commonly involved chromosomes. Balanced chromosomal translocation was therefore considered one of the causes of recurrent spontaneous abortion.

CONCLUSIONS

In summary, this study revealed the prevalence of chromosomal abnormalities to be 10.55% in male patients undergoing genetic counseling in Jilin Province, China, and among these chromosomal abnormalities, Klinefelter syndrome was found to be the most common abnormality. The findings of this study suggest that azoospermia and oligospermia may be a result of structural chromosomal abnormality. Chromosome 1 was most commonly found to be the chromosome involved in male infertility and balanced chromosomal translocation was shown to be one of the causes of recurrent spontaneous abortion. Chromosomes 4, 7, and 10 were found to be the most commonly involved chromosomes in male partners of women experiencing repeated abortion.

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